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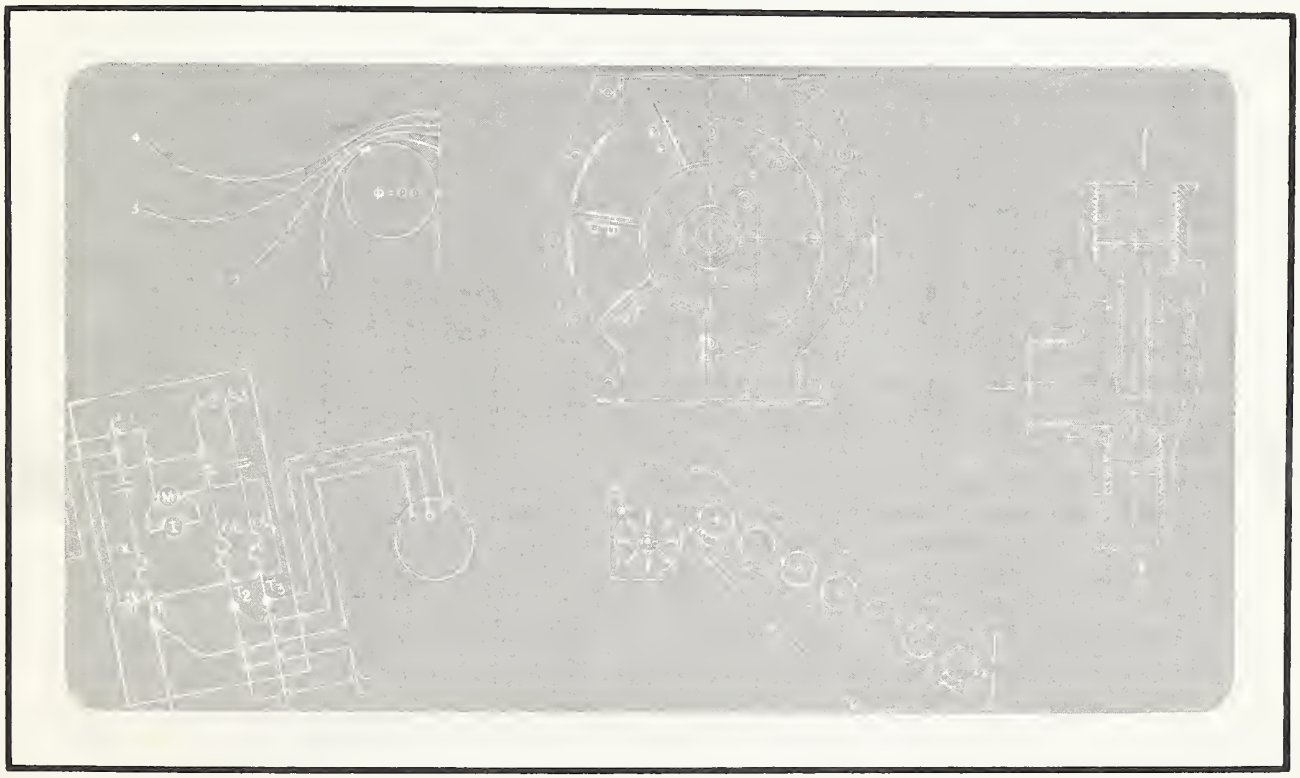
Mass Rearing of Horn Flies on a Host



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CONTENTS

ILLUSTRATIONS

TABLES

Mass Rearing of Horn Flies on a Host

By J. A. Miller, C. D. Schmidt, and J. L. Eschle[1]

ABSTRACT

As part of the Hawaii Horn Fly Project (1973–75), facilities and procedures were developed at the U.S. Livestock Insects Laboratory, Kerrville, Tex., to mass-rear *Haematobia irritans* (L.) on bulls, natural hosts of the fly. The equipment and procedures for holding the bulls; for collecting, mixing, and incubating the larval rearing medium; and for collecting and chilling the newly emerged adults are described. The potential of the system was demonstrated by the production of 21 million horn flies in 92 days, exceeding the goal of 1.5 million per week, but this production level could not be maintained. Declines in production were usually accompanied by the invasion of arthropods and nematodes, and when an infection of *Bacillus thuringiensis* Berliner persisted, production never recovered. In April 1974, a horn fly colony was established near Aiea on Oahu, Hawaii, using facilities and techniques similar to those developed in Texas. The mild, humid climate of Hawaii made it very difficult to maintain the desired environment in the facility, and parasitic mites continually interfered with production. The production goal of 1.5 million adult horn flies per week was never achieved. Suggestions for improving future mass-rearing programs are given. Index terms: *Haematobia irritans* (L.), Hawaii Horn Fly Project, insect-rearing equipment, insect-rearing facilities, insect-rearing methods.

INTRODUCTION

In the spring of 1973, the U.S. Livestock Insects Laboratory, Kerrville, Tex., began the Hawaii Horn Fly Project, to determine whether the sterile-male technique could be used in an integrated program with other control methods to eradicate a natural population of horn flies, *Haematobia irritans* (L.). We estimated that the study would require 1.5 million flies per week for sterilization and release.

Harris (1962) colonized horn flies in the laboratory and various modifications were reported (Harris et al. 1966, Schmidt et al. 1967, 1968). Later, Schmidt et al. (1974) developed a large-scale technique for a laboratory-type colony and reared the flies that Eschle et al. (1973) used to suppress a population of horn flies by the sterile-male technique.

Depner (1962) and Hargett and Goulding (1962) established and maintained colonies on cattle, and more recently, Berry et al. (1974) reported improved rearing procedures. Berry et al. extrapolated from an average daily production of approximately 25,000 pupae from a single steer to conclude that a six-animal facility should be capable of producing 1 million horn fly pupae per week. However, there was no previously developed system for producing the large number needed for our study.

In the production of insects for use in a sterile-

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male program, the quality of the insect is a primary concern. The adaptations necessary in colonization can result in an inferior insect or one which exhibits atypical behavior when released in nature. We tried to minimize these problems by rearing the horn flies for our test directly on cattle rather than using a total laboratory-rearing procedure. Furthermore, the colony was established from flies collected from the release site so as to reduce the possibility of strain differences. Our plans were to design facilities at Kerrville, where we could develop and refine procedures that would then be used in a similar facility on Oahu, Hawaii. The insects from both facilities were to be released after sterilization on Molokai.

In a host-reared colony of horn flies, the population is maintained directly on a bovine, and the females oviposit onto the manure droppings, as in nature. This manure is collected and incubated for a short period; then it is mixed with an artificial medium (Harris et al. 1967; Schmidt et al. 1968). The processed medium is incubated while the eggs and larvae develop. Pupae are normally extracted from the rearing medium by flotation. Some pupae are returned to the colony, and the remainder are sterilized by irradiation.

We used a single-animal unit to study production techniques and labor requirements before the development of full-scale facilities. Increased production was obtained by using improved larval medium, increased adult populations on the host, and improved procedures for handling materials. The results of these preliminary studies indicated that a six-animal facility should be capable of producing the desired 1.5 million horn flies per week.

SYSTEM DESIGN

FACILITIES

Because the Hawaii Horn Fly Project was temporary, the rearing system was developed in existing structures. The principal structure was an 8.4- by 12-meter building on a concrete slab that sloped (5 cm/m) from each sidewall to gutters located to either side of a 3-meter-wide raised section along the midline of the building. The lower 60 centimeters of the walls of the building were concrete. The upper 2.4 meters were metal and were insulated and lined with exterior-grade plywood. Darkened entrances were constructed on the concrete landings at both ends of the building so as to reduce the entry of unwanted insects such as house flies and borborids.

Within the building were areas for animal holding, medium processing, and early incubation. In one-half of the building six bulls were confined along one side in stanchions and stalls, each on an elevated 1.2- by 1.8-meter steel platform (fig. 1). The platforms were 30 centimeters above the floor at the rear and contained a 45-centimeter section of grating 30 centimeters from the rear edge (fig. 1C). The gratings covered a gutter positioned to catch urine. Ammonia odors from the urine were controlled by automatically flushing the gutter with 12 liters of water each hour. The platform was padded with rubber matting 1 to 2 centimeters thick for the comfort and safety of the bulls. The 1-meter distance between the side rails of each stall allowed the animals to lie down at will. Hay and water were provided in each stall. When the animals were correctly positioned on the platforms, they deposited manure onto plastic-lined, 60- by 90-centimeter, fiberglass trays on the concrete floor.

The other half of the building was used for processing the rearing medium and for early incubation. In one corner, manure containing horn fly eggs and awaiting mixture with rearing medium was stored on shelves. Nearby was the 0.2-cubic-meter concrete mixer used to mix the larval medium and manure (fig. 1D). The mixer interior was painted with an epoxy paint to facilitate cleaning and disinfecting. After the medium had been mixed, it was placed in disposable cardboard boxes and stored on mobile racks (fig. 2A). The stacked racks were then moved to the opposite corner for the first stage of incubation.

The building was air conditioned or heated to maintain 26° to 28° C and 60 to 70 percent relative humidity. Additionally, ventilation (two air changes per hour) was provided to reduce livestock odors and moisture in the air. Seven fluorescent lighting fixtures, each with two 40-watt lamps, were mounted on the ceiling, one to each side of each animal. These provided 270 lumens per cubic meter to the backs and upper sides of the animals, the areas preferred by the horn fly. The fixtures were totally enclosed to avoid trapping flies around the lamps. The building was windowless since natural light attracts the flies.

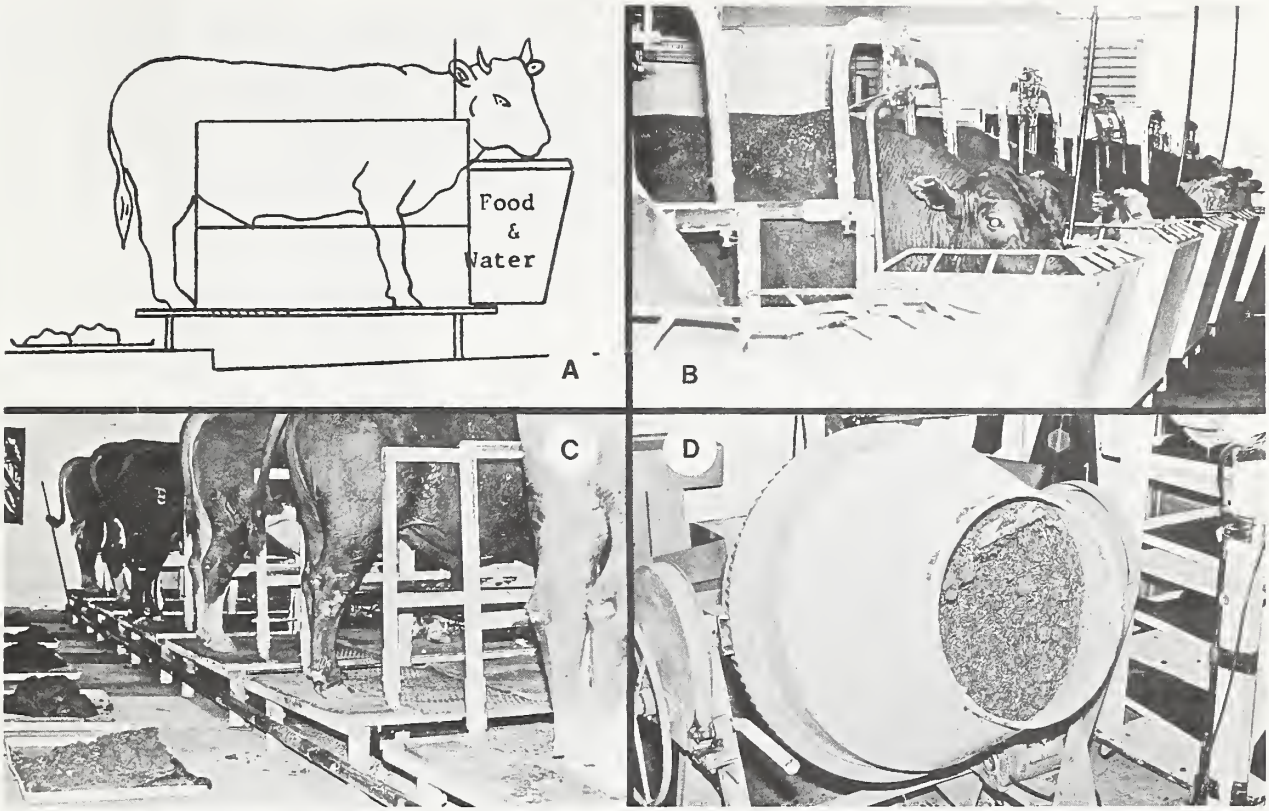

FIGURE 1.—Bull-holding unit. A, Schematic of animal on holding platform. B, Bulls in the stanchion with feed and water bunker. C, Position of bulls for manure collection. D, Mixing of larval rearing medium.

Normally, pupae are extracted from the rearing medium by flotation. However, this technique is time consuming, requires large volumes of water, and subjects the pupae to undesirable handling. We therefore developed a system of collecting and holding adult flies as they emerged from the rearing medium. The collecting unit was built in a second building that was also used for holding the medium during late incubation. The boxes of medium were held on mobile racks in a room maintained at 26° to 28° C and 60 to 70 percent relative humidity. In an adjacent room, a 1.2- by 2.4-meter walk-in cooler was constructed. The temperature in the cooler was maintained at 0.5° to 2.0° C, with a defrost every 6 hours. Two 40-watt fluorescent lights operated continuously in the cooler.

Two 1.2- by 1.2-meter chambers were constructed on each of the two long sides of the cooler. The interior of these chambers was painted flat black and sealed to eliminate light leaks. Thermostatically controlled ceramic heaters maintained 26° to 28° C in the chambers, and each chamber was ventilated with air (approximately 24 liters per second) from the surrounding room to remove accumulating moisture. A port (white PVC pipe, 10-centimeter-inside diameter) was installed through the upper rear wall of each chamber; each port led to collection containers in the cooler. Thus, as the flies emerged in the warm, dark chambers, they were attracted into the cooler by the light and were immediately immobilized by the cold environment (fig. 2B).

OPERATION

Adult horn flies released in the animal-holding room were able to feed on the bulls freely. Females deposited their eggs on the manure as the animals defecated onto the fiberglass trays behind each stall. Each morning the trays were removed and held 24 hours on open shelving to allow the majority of the eggs to hatch before the manure was mixed with the rearing medium. For mixing, the aged manure (about 14 kilograms per tray per day) was loaded into the concrete mixer, water was added, and the mixture blended for 2 minutes

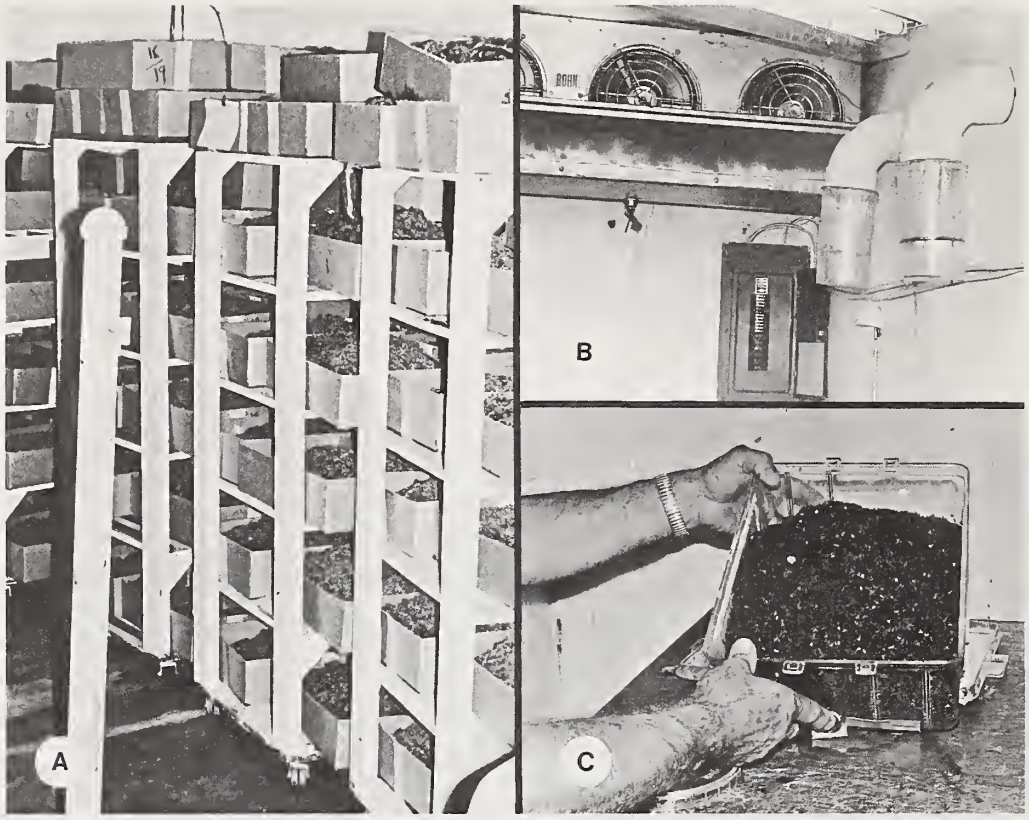

FIGURE 2.—Fly-collecting unit. A, Boxes of larval medium stored on mobile racks. B, Interior of chilling room. C, Chilled flies from collection containers.

before the artificial medium was added. The mixed larval medium was poured from the mixer into corrugated boxes, placed on the mobile racks, and removed to the opposite corner and held there 3 or 4 days (early incubation). A high-pressure pump was used in the daily cleaning routine of the animal-holding room. The animals' feet, the stalls, and the lower portion of the walls were carefully cleaned each day. The mixer, the fiberglass trays, and the plastic tray liners were washed after each use in a 0.1-percent sodium hypochlorite solution.

On the third and fourth day after the manure and medium had been mixed, the racks were moved to the incubation room in the second building. On the seventh day, one rack was rolled into each dark emergence chamber, and the doors were bolted. Adults usually emerged in the chamber over a 4-day period, from the 8th to the 11th day after mixing. Since emerging adults are both positively phototropic and negatively geotropic, they moved upward through the lighted port into the collection containers in the chilling room. Each morning chilled flies were collected (fig. 2C), sterilized, and prepared for shipping in their chilled state (Miller et al. 1977). Racks were removed from the chambers after the 11th day, and the chambers and racks were scrubbed with a sodium hypochlorite solution in preparation for reloading.

EVALUATION

Many of the problems we encountered in this six-animal unit convinced us that a large-scale facility could not be a simple multiple of the one-animal facility used in preliminary studies. However, this was the first attempt at such large-scale rearing of the horn fly, and the system's potential was demonstrated by the production of 21 million horn flies in 92 days. Thus, we exceeded the total of 1.5 million per week. (See the next section.) Also, production labor requirements did not exceed 20 man-hours per day, although many processes were manual.

The holding facilities and equipment were developed with consideration for the safety of

both bulls and workers. The bulls were rotated in and out of the system to protect their health, and we did not have to remove an animal because of injury. Some bulls remained in the stalls more than 3 months without noticeable detriment.

A ventilation rate of two air changes per hour and hourly flushing of the gutter beneath the animals was sufficient to avoid odor accumulation. When livestock and ammonia odors were excessive, the flies tended to become disoriented, and rested on the walls and ceiling rather than on the animals, and the workers became uncomfortable.

The procedures for handling materials were adequate for the desired production level; however, a larger system would require increased mechanization. The manure produced by each animal each day, approximately 14 kilograms, was easily mixed as a single batch in the concrete mixer, and the handling of all six batches usually took less than 1.5 hours.

We found that using corrugated boxes for holding larval rearing medium eliminated the need for washing and sterilizing the rearing pans. The porous boxes allowed water to drain, fermentation gases to escape readily, and heat to dissipate during incubation. These advantages were enhanced by placing the boxes on slatted shelving and providing forced-air circulation around them. Boxes 30 by 46 by 13 centimeters deep were most convenient for handling and storage, and 200-pound-per-square-inch burst strength was sufficient to avoid rupture during handling. The mobility of the storage racks facilitated handling of the larval medium, but in a larger facility, an overhead rail system might be desirable.

The greatest labor savings resulted from the development of the system for collecting emerging adults, which eliminated the need for extracting the pupae from the larval medium. Furthermore, collection of adults enabled us to take advantage of the earlier emergence of females (Harris et al. 1971) and thereby control the male-female ratio of flies returned to the colony. Chilling of the flies reduced debilitation during holding and shipping (Miller et al. 1977).

A lagoon 8 by 8 by 1.2 meters deep was sufficient to handle the water waste from the animal facility. We encountered no problem in disposal of the larval medium after adult emergence since gardeners were willing to haul this material away.

PRODUCTION TECHNIQUES

Larval Rearing Medium

Before we activated the six-bull facility, we used the single-steer unit described by Berry et al. (1974) to study techniques for increasing production. Improvement of larval rearing medium to achieve greater survival was one of the first goals. Harris et al. (1967) developed the first dry mix for larval medium, and variations of it have been reported (Schmidt et al. 1968, Berry et al. 1974). Initially, we used a dry mix containing (parts by weight) 40 ground bagasse, 8 whole-wheat flour, 6 fishmeal (65 percent protein), and 1 sodium bicarbonate (baking soda). In tests of media containing 75 percent moisture, the ratio of 2 parts feces to 1 part dry mix produced more pupae than produced by media with ratios of 3 to 1, 4 to 1, or 5 to 1. Also, more pupae were produced in the 2-to-1 mixture when the moisture content was 70 percent rather than 75 or 80 percent, but it was more difficult to separate the pupae from the 70-percent-water medium because larger quantities of material floated with the pupae. Thus, the 75-percent-moisture level was preferred, but the use of porous corrugated boxes as rearing containers (enamel pans had been tried) required a medium containing more water. After comparing the original medium—2 parts feces, 1 part dry mix and 2.6 parts water (75 percent)—and mix ratios of 2:1:3 (76.7 percent water) and 10:3:10 (78.3 percent water), the latter mix was found to be more productive and was used for the duration of the project.

Bovine feces used in rearing horn fly larvae should contain about 80 percent moisture and form a glossy mound when dropped from the animal. If the feces are too soft, they run; if too dry, they break when dropped. The bulls were fed alfalfa cubes and Coastal bermudagrass hay at rates on which they each produced approximately 14 kilograms of feces per day. Before aging the egg-infested feces for 24 hours, we spread the feces over the entire tray to reduce suffocation of eggs and larvae.

Maintenance of Flies on Hosts

Berry et al. (1974) maintained a population of approximately 6,500 flies on a steer by returning

1,000 pupae per day to the colony. Our studies indicated that a population of 10,000 flies could be maintained by returning 2,000 pupae per day. A return of 3,000 pupae per day resulted in a population of approximately 12,000 flies. In each case, mean daily production for a 2-week test at each population level with the 2:1 (75 percent-moisture) larval medium described above resulted in four pupae per adult fly on the steer.

The steer became too nervous and irritable when the population of flies exceeded 10,000, and so we stanchioned a bull to determine its reactions. With more than 12,000 horn flies, the bull was less irritated than the steer with 10,000. And in a 2-week production trial, daily production of pupae averaged more than 45,000, further confirming that a daily production of 40,000 or more pupae from 1 animal was possible. In June 1973, rearing began with six stanchioned steers and the larval medium described above. When the horn fly population exceeded 5,000 on each steer, the irritability of each was compounded. After one steer began acting up, all would likewise react to the flies, and their nervousness prevented production of the desired quality of feces. To solve the problem, the steers were replaced by bulls, British breeds or crosses weighing 400 to 500 kilograms. Rather than being clipped (Berry et al. 1974), the bulls were groomed to remove fly feces, scabs, and scales of skin. A bull was replaced with another on the basis of production of flies, condition of skin, soreness or swelling, and freedom from infection, fever, or other ectoparasites.

Until the adult collection facility was completed, pupae were separated from the larval medium, and the adults emerging from pupae in the bull room infested the bulls. By November 1973, we had changed from native Texas flies to a strain of horn flies colonized with pupae from Molokai, Hawaii. Then, new adults were released in the bull room daily to maintain fly populations.

Harris et al. (1968, 1971) reported that one male horn fly mated with an average of five females and that more females than males

Table 1.—Number of horn flies released on bulls and produced, sex ratios, and production of bull feces, Kerrville, Tex., 1975

Production week (beginning on date medium mixed)	Flies released on bulls		%♀ on bulls	Flies produced			
	Number	%♀		Feces collected (kg)	Number[1]	%♂	No./kg feces
2/2	94,336	67	87	543	1,494,847	43	2,753
2/9	92,921	68	89	495	1,620,328	41	3,273
2/16	116,030	75	86	511	1,456,997	39	2,851
2/23	98,789	71	85	558	1,234,615	48	2,213
3/2	106,231	67	92	507	1,541,231	42	3,040
3/9	122,135	72	86	544	1,850,269	36	3,401
3/16	104,292	76	73	542	1,614,271	48	2,978
3/23	93,518	76	88	534	1,205,111	47	2,257
3/30	93,028	64	...	483	949,458	44	1,966
4/6	107,302	65	82	493	539,360	45	1,094
4/13	109,286	60	85	522	1,041,895	42	1,996
4/20	136,754	67	85	520	1,107,868	50	2,131
4/27[2]	127,178	63	89	534	1,497,498	42	2,804
5/4[2]	125,890	76	87	578	1,898,776	50	3,250
5/11	126,927	67	90	555	2,279,377	45	4,107
5/18[2]	161,904	72	93	438	2,023,534	42	4,620
5/25	132,391	76	92	475	2,420,724	43	5,096
6/1[2]	162,179	75	93	509	2,256,100	41	4,432
Average	117,283	70	87	519	1,556,237	43	2,999

[1] Includes flies collected from boxes of larval medium used for sampling purposes; these flies were ultimately returned to their respective groups. Flies were collected 7 to 11 days after mixing.

[2] Some of the daily releases included flies collected from individual boxes of larval medium.

emerged during the first 24-hour period of eclosion. We assumed that the production potential would be greater if the released flies were 70 to 90 percent female instead of 50 percent. Thus, daily releases of the first 2,000 to 3,000 emerged flies per bull were made by weighing a predetermined amount, for example, 45 grams, and releasing them in the bull room after cleanup. As the flies warmed up, they were free to infest the bulls.

MONITORING PROCEDURES

To determine the production level and identify factors that might cause changes in production, we kept daily records of the number and sex ratio of new adults released on the bulls, the weight of feces collected from each bull, and the total number, sex ratio, and weight of adults collected from each lot of rearing medium. To determine the number of flies from each lot of medium, we weighed the collection of adults from each compartment each morning and removed random samples of 100 flies, which were weighed for calculation of average weights and sorted for determination of sex ratios. Then, we calculated the total number of flies from each lot and combined all the flies for further handling.

Approximately once each week, 100 eggs were collected from fresh feces (less than 6 hours old) of each bull, held 48 hours at 27° C on wet filter paper in a petri dish, and checked for hatch by counting empty eggshells. Then, as the medium was prepared with these feces, we checked the eggs again by first dipping 50-milliliter samples of the slurry (feces and water mix) from each bull. From a subsample of the slurry containing 10 grams of feces, we removed the eggs and egg cases by mixing the subsample with 1,000 milliliters of water and 50 grams of baking soda in a 1-liter sedimentation funnel, pouring the supernatant into a cloth-covered Buchner funnel to remove the excess water, and transferring the eggs from the cloth (and any that floated afterward in the funnel) to wet filter paper in a petri dish. Twenty-four to forty-eight hours later we counted all the eggs and the hatched eggs to estimate egg and larva production.

We estimated the number of pupae produced by removing puparia from a sample of spent medium of each bull and by counting the number from which adults emerged. With the percentage emergence and total number of collected adults, we calculated the number of pupae.

To estimate the production level and comparative efficiency of the bulls, we collected adults from individual boxes of the larval medium from which eggs had been sampled. These boxes were placed under fiberboard pyramids fitted with 1,400-milliliter screw-top containers with inverted funnel traps to capture the emerging adults.

The sex ratio of the horn fly population was estimated by examining collection of flies from each bull. We found no means for confidently estimating the number of flies on six bulls in one room.

RESULTS AND DISCUSSION

Horn flies were reared on stanchioned bulls from August 1973 through December 1975. We frequently changed rearing procedures through 1974 to increase the production level. By September 1974, most of the procedures were standardized; however, the production level was never consistent. From February to June 1975, production averaged about 1.5 million adults per week, of which 43 percent were male (table 1). The mean weekly production for a 63-week period (September 1974 to November 1975) was approximately 860,000.

We released 2,800 new adults per bull per day, of which 70 percent were female, but populations of adults on the bulls averaged approximately 87 percent female so females survived longer than males. During the weeks of lowest production, it was necessary for us to exchange several bulls. The weekly weight of feces fluctuated from 438 to 578 kilograms.

By checking eggs, we were able to evaluate fertility and fecundity of the female flies and the effects of handling egg-infested feces on survival of the eggs. The flies on the six bulls were equally fertile because approximately 90 percent of the eggs off of fresh feces from each bull hatched from July 1974 to September 1975. Only 82 percent of the eggs hatched when they were collected after the feces and water were mixed. The lower hatch could have been caused by suffocation in the feces, the quality of feces, or by the agitation of the mixer. During the summer of 1975, when the egg hatch from fresh feces and slurry was about the same, the quality and consistency of feces were most uniform.

The average daily egg production for the 15-month period ranged from 101,000 eggs per day for bulls in position 2 to 118,000 eggs per day for those in position 6; however, this difference was

not statistically significant at the 5-percent level. Collections of adults from individual boxes of larval medium did no more than confirm these production trends and allowed us to evaluate the comparative efficiency of production of the individual bulls. The average number of emerging adults per box was usually higher in the collection compartments than under the sampling cones.

From larval medium for which the number of eggs was previously estimated, we calculated the mean efficiency of production (percentage adults produced from eggs) as less than 20 percent. Since survival of eggs was approximately 28 percent, a serious loss was occurring in either the larval stage or pupal stage or both. Table 2 reports the estimated percentage survival of eggs, larvae, and pupae (15 observations). The data clearly show that larval survival was the lowest.

Production reached or exceeded the expected level of 1.5 million adults per week for a few months in 1975. The level of production was always inconsistent, although the rearing procedures during the final stages were standardized. We could not simply increase the number of bulls and increase horn fly production proportionally because there were several uncontrollable factors. For example, each bull was different; thus, the environment for the adult flies as well as the environment for the larvae in the feces was variable. Also, low production during March, April, September, and October 1974 and from August to December 1975 was accompanied by invaders. In the spring of 1974, house flies, *Musca domestica* L., and other small Diptera infested the larval medium. In the late summer and fall of 1974, *Pygmephorus athiasae* Wicht (Acarina: Pyemotidae), *Leptocera elegans* Spuler (Diptera: Sphaeroceridae), *Milichiella lacteipennis* (Lowe) (Diptera: Milichiidae), and a phoretic nematode infested the larval medium. Then, in the latter part of 1975, small Diptera, nematodes, and a Histiostoma (Acarina: Anoetidae) infested the medium and new adults. For 2 consecutive years, declines in production efficiency occurred in the late summer and early fall months. These declines followed the spring and summer invasion of arthropods. *Bacillus thuringiensis* Berliner also contaminated the larval medium in 1975. Several types of this pathogen were isolated from various life stages of horn flies, bull feed, bull feces, and mites (R. E. Gingrich, personal communication). Because the source of this contaminant was not determined, we stopped rearing in early September 1975 in order to sterilize the facilities and to bring in new bulls. Nevertheless, the organism reappeared and persisted, so rearing was terminated in December 1975.

Table 2.—Survival of horn fly eggs, larvae, and pupae based on estimates of hatch, pupation, and emergence, Kerrville, Tex., 1975.

Date medium mixed	Survival (%)		
	Eggs	Larvae	Pupae
8/7	69	16	67
8/14	67	11	69
8/22	86	21	50
8/28	76	18	54
9/2	76	6	78
9/25	80	51	97
10/2	83	44	96
10/9	83	28	96
10/16	80	23	96
10/24	84	12	95
10/31	86	5	79
11/6	71	4	57
11/12	75	5	8
12/1	77	17	64
12/15	87	25	72

ESTABLISHMENT OF HAWAIIAN COLONY

On the basis of the research described in preceding sections, a horn fly colony was established on Oahu, Hawaii, in April 1974. The production was to be used for an eradication experiment on Molokai.

The facility was located at the Hawaii State Quarantine Station near Aiea. Six bulls were stanchioned in a 67-square-meter room leased from the Hawaii State Department of Agriculture. Three stanchions faced the east side of the room and three faced the west side. This bull room, which had continuous fluorescent lighting, was maintained at 26° to 28° C and 60 to 70 percent relative humidity, and ventilated at a rate of two air changes per hour, as in Texas. Care of the bulls included daily feeding of alfalfa cubes and grass hay, twice weekly feeding of a mineral salt mix, and daily grooming. Daily releases of 13,000 to 15,000 horn flies colonized from the Molokai strain established at Kerrville maintained an infestation of approximately 10,000 per bull.

For the larval medium, the egg-infested manure was collected and stored for 28 hours in the bull room rather than for 24 hours as in Texas. Moist bagasse was obtained in bulk from Oahu Sugar Co., dried overnight with a grain dryer (850,000-Btu), ground through a 6-millimeter screen, and stored in plastic bags. The dry mix, which differed from that used in Texas, was prepared with 8 parts (by weight) of whole-wheat flour, 2 parts of fishmeal (60 percent protein), and 1 part of bicarbonate of soda. The larval medium was prepared daily by mixing, for 1 minute in a 0.2-cubic-meter concrete mixer, 14 kilograms of stored manure with an equal part of water and 0.1 part dry mix; 0.4 part more of bagasse was added, and mixing was continued an additional 3 minutes. The medium was then held in corrugated paper boxes on slotted shelves that were ventilated with a 900-liter-per-second fan. The mixing room was air-conditioned to maintain it at 26° to 28° C and no more than 85 percent relative humidity. The larval medium was held 5 or 6 days in an adjacent building, then moved into a six-compartment facility for the collection of emerging adults. These adults were processed as in Texas and shipped to Molokai (Miller et al. 1977).

The bull room, stanchions, mixing room, mixer, and emergence chambers were thoroughly cleaned daily with brushes and a solution of 30 milliliters of detergent (A-33) and 30 milliliters of 6 percent sodium hypochlorite per 3.8 liters of water. Additionally, the bull room and mixing room were usually sprayed twice weekly with acaricides to combat an infestation of mites; this spraying was done more frequently when infestations were severe. Emergence chambers were sprayed with acaricides before and after being cleaned and again just before being filled with boxes of rearing medium. Initially, sprays of 0.1 and 0.3 percent tetradifon (4-chlorophenyl 2,4,5-trichlorophenyl sulfone) were used. Later, we used 0.1 and 0.05 percent dicofol [4,4′-dichloro-α-(trichloromethyl)benzhydrol]. No ill effects on the horn flies were observed after the application of either acaricide.

Monitoring similar to that in Texas was done periodically to obtain some indication of production efficiency. During production weeks 16 to 43, we estimated the percentage of pupae that eclosed by counting the adults that emerged from 50 pupae from each of 2 boxes of rearing medium held on the shelf in a cloth-covered 120-cubic-centimeter plastic cup. Once each week, during the last 9 weeks of production (weeks 42 to 50), we estimated the number of eggs laid and hatched from 1-day collections of manure. The adult flies that emerged from the sample were used to calculate total adult production (percentage of eggs that developed into adults).

Soon after the Hawaiian facility was established, a mite infestation developed and remained a problem throughout the program. Two species were identified: *Macrocheles subbadius* (Berlese), family Macrochelidae, and *Pygmephorus mesembrinae* Canestrini, family Pyemotidae. Mites were occasionally observed on manure in the bull room and were seen frequently in boxes of rearing medium and on the storage shelves in the mixing room. During the 4 days of fly emergence from the rearing medium, mite populations increased noticeably, and during periods of extreme infestations, the mites congregated in piles in the corners and crevices and on the floors of the emergence chambers. Both species are phoretic, so a continuous cycle of reinfestation was maintained when adult flies infested with mites were returned to the bulls. Axtell (1964) studied the phoretic relationship of several manure-inhabiting Macrochelidae and the house fly, *Musca domestica* L., and found that among the species collected from manure inside barns, *Macrocheles subbadius* and *M. muscaedomesticae* (Scopoli) are phoretic. Mites were probably introduced into the Hawaiian colony early in the program by an infestation of house flies that was observed at approximately the same time as the first mites were found on horn flies collected from the emergence chambers.

We conducted two cursory experiments with macrochelids in the Hawaiian colony. Horn fly eggs were placed on strips of moist filter paper in 2-dram shell vials to which varying numbers of mites were added (Axtell 1961), and percentage hatch was determined after 24 hours. We also determined the number of adults that developed from eggs exposed to mites in 180-cubic-centimeter cups that contained 80 grams of rearing medium each. Also, to obtain an infestation index, we examined the daily samples of 100 flies from each emergence chamber and determined the percentage infested with mites; no attempt was made to separate mites by family, although the macrochelids appeared to be predominant.

Average weekly production of horn flies for 50 weeks (October 1974–September 1975) is sum-

marized in figure 3. Although operation of the Hawaiian facility was begun July 1974, initial production was very low (not shown in fig. 3). One problem was that a standard rearing medium was difficult to develop since bagasse obtained from the sugar mill varied in quality and moisture. Finally in October (production week 1), when we began drying the bagasse, we were able to prepare a rearing medium comparable to that used at Kerrville. Nevertheless, average horn fly production for the first 14 weeks was only 181,300 flies per week. A serious infestation of house flies and, subsequently, mites developed in the colony. To reduce the exposure to house flies, the boxes of rearing medium were held in the bull room the first 4 days of incubation and then transferred to a holding area near the emergence chambers. However, this caused crowding in the bull room, making cleaning difficult and necessitating several modifications. The mixing room, previously a screened enclosure at the entrance to the bull room, was therefore enclosed and air-conditioned to increase security against house flies and to provide space for the medium during incubation. The bull room, mixing room, and emergence chambers were routinely sprayed with acaricides (tetradifon or dicofol), and a vigorous sanitation procedure was established. Samples of each group of flies that emerged were examined for mites, and only flies free of infestation were returned to the bulls.

These modifications were completed by week 12 and production increased to 687,400 flies per week and remained at that level for 14 weeks (weeks 15-28). Although production exceeded 1 million flies during week 26, the goal of 1.5 million per week was not reached. During week 20, high mortality of adult flies on the bulls resulted in a sharp decline in production that was attributed to the presence of dichlorvos resin strips installed in another part of the building. Removal of the strips during week 20 was accompanied by an immediate recovery in adult survival and an increase in production.

Production for weeks 29 to 42 declined to an average 569,000 flies per week, so several modifications were attempted. There was no advantage to incubating manure 48 hours rather than 28 hours before mixing. Production from medium held in boxes with 10-centimeter sides was essentially the same as that from the standard boxes (14-centimeter). Likewise, varying the amount of water in preparing the rearing medium had no effect on production. Neither did adding water 24 hours or 48 hours after mixing nor lining the boxes with plastic to reduce evaporation.

Production continued to decline. During weeks 43 to 50, it dropped to an average 385,300 flies per

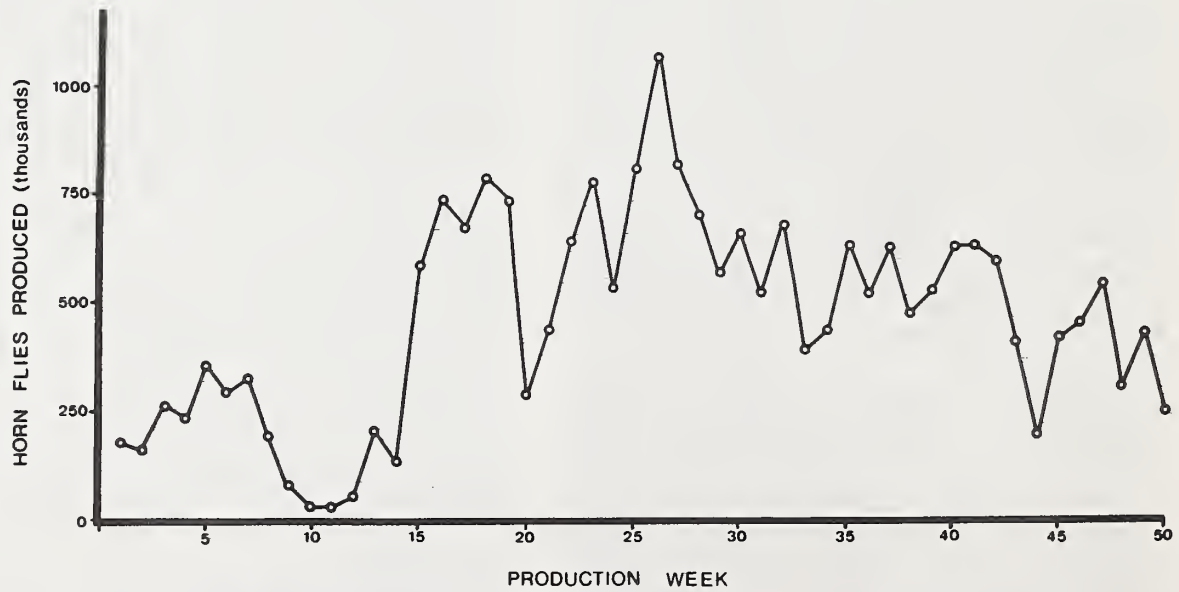

FIGURE 3.—Average weekly production of horn flies, Oahu, Hawaii, Oct. 6, 1974–Sept. 21, 1975.

week. At the end of 50 weeks, the Hawaiian facility was closed without having reached the production goal of 1.5 million flies per week. Nevertheless, results at Kerrville indicated that such a high level of production was possible with the rearing methods used.

Data from the last 9 weeks of production (weeks 42 to 50) provided some indication of weak points in the system. We estimate that, during this period of low production, colony flies laid an average 632,549±37,860 eggs per day (±SD, $n=10$), of which an average 71 percent hatched. Average production from rearing medium that contained these eggs was 43,957±8,259 adult flies per day (±SD, $n=10$) or an average yield of 7 percent. Average eclosion of pupae sampled during weeks 16 to 43 was 63±2 percent (±SD, $n=28$). By extrapolation (data for pupae were only available for the first 2 weeks of the 9-week monitoring period) and calculation, we estimated a larval mortality rate of approximately 84 percent. Similar data were not available for periods of maximum production in the Kerrville colony (over 2 million flies per week), but the average yield from an estimated 769,000 eggs per day (87 percent hatch) was 40 percent (estimates of pupal eclosion were not available).

Although egg fertility and pupal eclosion rate were somewhat low, the primary factor limiting production in the Hawaiian colony was associated with the larval stage. Similar conclusions were reached in evaluation of the Kerrville facility. There were indications that infestations of mites influenced production. The macrochelids that were predominant in Hawaii probably caused mortality to immature stages of the flies and may also have been detrimental to adults (F. H. Haramoto and F. J. Radovsky, personal communication). In Axtell's laboratory tests (1961), *M. subbadius* destroyed eggs and first-instar larvae of the house fly at a rate of 1.2 per day per adult female mite. In another experiment, when Axtell (1963) put 400 macrochelids in an indoor cage containing manure infested with 20,000 fly eggs, production was reduced 86 to 98 percent. Our own limited vial tests in Hawaii showed that 100 percent of the horn fly eggs were destroyed at ratios of two or more macrochelid mites per egg and that mortality of immature horn flies was 63 and 85 percent when mites were exposed to eggs at ratios of 1 : 1 and 4 : 1, respectively. There was further evidence of a relationship between the degree of mite infestation and the production of adult flies. During the first 13 weeks, when average production was approximately 181,000 flies per week, we estimated that 7 percent of the emerged adult horn flies were infested. After we began using acaricides and other measures to control mites and infestations averaged only 0.1 percent (weeks 14 to 33), production averaged 644,000 flies per week. During weeks 19 to 33, when the highest production was reached, no mites were found on newly emerged flies. During the last 17 weeks, production was 459,000 flies per week, and an average 0.5 percent of flies were infested with mites.

In addition to mites, other less defined factors may have contributed to the low production in Hawaii. The source and method of handling dry bagasse were different from those used at Kerrville. Also, we were not always able to maintain the environmental conditions maintained in the Kerrville facility. The tropical climate in Hawaii made it difficult to control temperature and relative humidity and, at the same time, provide adequate ventilation using outside air. Because of the year-round mild climate, invasion by house flies and other arthropods was a constant problem in Hawaii, but essentially not a factor during the winter months at Kerrville. Unidentified pathogens may also have limited horn fly production in Hawaii since *Bacillus thuringiensis* caused a sharp drop in production and, eventually, the termination of rearing at Kerrville. This and other disease organisms have often been the cause of mortality of immature stages of horn fly colonies (Kunz and Eschle 1971).

When maximum production was obtained during weeks 18 to 42 in Hawaii and Kerrville, we were able to utilize the combined production from both colonies to successfully complete an experiment in which the release of sterile flies, along with the use of an insect growth regulator, eliminated a native horn fly population from a group of about 750 cattle on the east end of Molokai (Eschle et al. 1977).

CONCLUSIONS AND RECOMMENDATIONS

In addition to providing horn flies for the Hawaii Horn Fly Project (Eschle et al. 1977), this study accentuated the problem areas in mass rearing of the horn fly. Further research will be required before embarking on large-scale experiments or areawide pest-management programs

requiring mass releases. Future facilities should be housed in a single building with partitioned sections for the various production stages; this will reduce labor requirements, simplify sanitation procedures, facilitate environmental control, and improve security against the invasion of arthropods. Although the Kerrville facility operated with only four emergence chambers, we recommend a minimum of six, since a change of 2° or 3° C can alter the emergence period and the schedule for loading each chamber. Also, we recommend a prefabricated walk-in cooler with 10 to 15 centimeters of rigid urethane insulation and aluminum or plastic interior and exterior walls for the chilling room. Each emergence chamber should have a floor drain for ease of cleaning. A technique for evaluating the vigor of new adults should be developed. Perhaps most important, ways should be sought to increase larval survival and these may include determination of key nutrients, roles of associated micro-organisms (both beneficial and detrimental), effects of fungi and molds, effects of invading free-living arthropods and nematodes, and optimum microenvironment.

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